ON THE POPULATION BIOLOGY AND NATURE OF DIAPAUSE OF LABIDOCERA AESTIVA (COPEPODA: CALANOIDA)¹

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Knowledge of the seasonal distribution patterns of many marine copepods led Fish and Johnson (1937) to postulate that these species might produce resting eggs as an adaptive mechanism to insure survival at times of the year unfavorable to a planktonic existence. It was not until 1972 (Zillioux and Gonzalez, 1972) that conclusive results demonstrating the production of resting eggs by a marine copepod were obtained. During the last decade, it has become increasingly evident that the production of resting eggs by temperate, inshore marine copepods is a widespread event in this group (Zillioux and Gonzalez, 1972; Kasahara, Onbe and Kamigaki, 1975; Landry, 1975a; Grice and Gibson, 1975, 1977; Grice and Lawson, 1976; Uve and Fleminger, 1976). These studies have documented the existence of resting eggs in bottom sediments (Kasahara, Uye and Onbe, 1974, 1975; Kasahara, Onbe, and Kamigaki, 1975; Grice and Gibson, 1975, 1977; Uye and Fleminger, 1976) and demonstrated their production by females collected from the plankton (Grice and Gibson, 1975, 1977; Grice and Lawson, 1976; Uye and Fleminger, 1976; Zillioux and Gonzalez, 1972). These investigations have shown that physical factors such as temperature, light, salinity, and oxygen concentration affect the maintenance and termination of the resting egg. The events which actually trigger the induction of resting egg production are still poorly understood. The elucidation of factors which influence the induction, maintenance, and termination of copepod resting eggs is necessary to achieve a better understanding of the biology, evolution and distribution patterns, both in time and space, of neretic temperate-boreal copepods which are key elements in the food web.

Labidocera aestiva is a large calanoid copepod reported to occur in coastal waters from the Gulf of St. Lawrence to the Gulf of Mexico (Wheeler, 1901; Grice, 1956; Fleminger, 1957; Cronin, Daiber and Hulbert, 1962; Anraku, 1964; Van Engel and Tan, 1965; Bowman, 1971; Fleminger, 1975). The populations occurring north of Cape Hatteras are seasonally abundant in the plankton, with maximum numbers of nauplii, copepodites, and adults occurring in the summer and fall, and disappearing by mid-December. Grice and Gibson (1975) and Grice and Lawson (1976) have demonstrated the production of resting eggs by females of L. aestiva collected in the field and their presence in bottom sediments.

During the summer-fall period in 1977, a preliminary investigation of subitaneous and resting egg production by *L. aestiva* from Vineyard Sound, Massachusetts was conducted. This work indicated that the production of resting eggs commenced in early September. The surface water temperature was 19.5° C. The data presented in this paper document the seasonal variation in subitaneous and resting

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FIGURE 1. Trends in body size (cephalothorax, solid square, total length solid circle), percent hatch (open square), and surface water temperature (open circle) for the sampling period July 7, 1978 to December 6, 1978.

egg production during the summer-fall period in 1978. It is shown that the proportion of subitaneous eggs produced is greater during the summer, decreases markedly in September, and persists as a small percent throughout the fall. Moreover, the major change from subitaneous to resting egg production precedes the initiation of increasing adult body size and decreasing surface water temperatures by 2 weeks. It is demonstrated that resting eggs which are chilled at 5° C for a minimum period of 30 days, will hatch synchronously within 2 days when warmed to 21° to 23° C. Comparable eggs kept continuously at 19° to 23° C will hatch over a longer period of time and not synchronously. This paper discusses the significance of these findings as they relate to diapause induction, maintenance, and termination, as well as the population dynamics of *L. aestiva*.

MATERIALS AND METHODS

Specimens of *Labidoccra acstiva* were collected from July through December at weekly intervals from a 1 to 2 m depth in Vineyard Sound, Massachusetts by towing a 1-m, 243- μ mesh net for a 10 min period. Adult males and females were removed from these two samples by pipette. Twenty individuals of each sex were preserved in 5% buffered formalin each week, except for collections in late fall when very few adults were present. Two measurements of body size were obtained for each preserved specimen (*i.e.*, cephalothorax, and total length) using a stereomicroscope and ocular micrometer. During the summer months, eggs were collected by placing 10 females together in separate 100-ml dishes. *Gymnodinium nelsoni* was added as food, and the dishes were placed overnight in an incubator at 19° C, with a 12 to 12-hr photoperiod. The following morning eggs were removed by pipette and counted. The percent hatch was determined for an aliquot (100–120) of these eggs. During the summer, eggs usually hatched within 2 days. The same procedure of collection, counting, and determination of percent hatch was followed for fall eggs. Fall eggs which did not hatch within 4 days were placed in filtered sea water (glass fiber) in 75-ml screw-top glass jars. The jars were incubated at 19° C or refrigerated at 5° C. At the appropriate time, the refrigerated jars were warmed to 21° to 23° C and percent hatch determined. At the same time, the percent hatch in the jars kept at 19° C was determined.

Results

The trends in body size, percent immediate hatch, and surface water temperature for the six-month period between July and December 1978 are shown in Figure 1. The standard deviations for body size ranged between 0.01 and 0.17. For each sex, changes in cephalothorax length paralleled total length. At each census throughout the sampling period, female body size was larger than male body size. An inverse relationship was observed for body size and surface water temperature for both females and males, and is depicted for adut females in Figure 2a. The smallest adults were collected during July and August when water temperatures ranged between 20° to 22° C. A marked increase in size began in September reaching a maximum in late November.

The percent immediate hatch (*i.e.*, within 2–4 days) of eggs collected from grouped females was greatest from July to mid-August, ranging between 74 and 93%. Subsequently the percent immediate hatch began to decrease and was observed to vary between 6 and 27% from mid-September to early December. The marked decrease in the portion of eggs hatching immediately began in late August, and preceded the initial drop of surface water temperatures to below 20° C (Figs. 1, 2b) and the time at which body size began to increase. No obvious difference in total egg production was observed, although differences may exist.

The percent hatch of eggs incubated at 19° or 5° C and then later warmed to room temperature $(21^{\circ}-23^{\circ}$ C) is shown in Table I. The data presented are for eggs laid within 1 day of collection for five samplings between early September and late November. In general the percent hatch of eggs kept at 5° C and then warmed to 21° to 23° C was higher than those incubated at 19° C. Moreover, the hatch of the 5° C eggs occurred synchronously within two days of being warmed to 21° to 23° C. It was determined from periodic examination of the jars held at 19° C that non-synchronous hatching occurred throughout the incubation interval. Some of the unhatched eggs at 19° C appeared dead (brown, granulated interior), whereas others appeared viable (greenish hue).

DISCUSSION

An inverse relationship between body size and temperature has been demonstrated for a number of marine and freshwater copepods (Coker, 1933; Marshall,



FIGURE 2. Scatter diagrams of female total body length (a), and percent hatch (b) versus surface water temperature at the time of collection.

Nicholls, and Orr, 1934; Aycock, 1942; Deevey, 1960a, b; McLaren, 1965; Deevey, 1966; see review in Hutchinson, 1967; Durbin and Durbin, 1978). The seasonal pattern of size variation reported herein for *Labidocera aestiva* from Vineyard Sound is similar to that reported by Deevey (1960a) for another population of the species from Delaware Bay studied in 1930. However, the patterns are shifted in time. The smallest individuals are found during August in Vineyard Sound and September in Delaware Bay. Deevey (1960a) reports that the average surface water temperature in Delaware Bay was 22° C during September. This value

TABLE I

Date collected	Date incubated		Period of incubation	(7 - 11 - 4 - 1
	5°	19-23°	(days)	% Hateh
Sep 27	Oct 2		75	96
Sep 27		Oct 2	75	61
Oct 5	Oct 10		111	95
Oct 5		Oct 10	111	42
Sep 7	Sep 11		58	83
Sep 7	Î	Sep 11	58	2
Oct 19	Oct 23		126	95
Oct 19		Oct 23	126	55
Nov 21	Nov 27		87	95
Nov 21		Nov 27	87	95

Percent hatch of incubated $(19^{\circ}-23^{\circ} C)$ and refrigerated Clutch 1 eggs after warming to $21^{\circ}-23^{\circ} C$, for five samplings between early September and late November.

corresponds to temperatures recorded for Vineyard Sound in August (Fig. 1). The pattern, however, varies from year to year within an area, inasmuch as the smallest individuals during 1932 in Delaware Bay were collected in early August, when temperatures were then highest. It is obvious that body size is correlated more with the surface water temperature than with the time of collection.

In the present study, there was no evidence of discrete generations for L. aestiva in Vineyard Sound. Breeding appeared to be continuous throughout the summer and fall. Deevey (1960a) suggested that spawning was continuous during the summer for the Delaware Bay populaton, but that a break occurred between early August and September, resulting in a burst of larger individuals late in October. Moreover, it was suggested that the offspring of the large-sized fall individuals would not mature until the following year. More recent studies of L. aestiva from Vineyard Sound (Grice and Gibson, 1975; Grice and Lawson, 1976) have demonstrated that the offspring of the fall individuals survive the winter as resting eggs, and hatch the following spring. It is probable that the same life history pattern is common to the Delaware Bay population.

In Vineyard Sound, the population continues to breed at temperatures as low as 8.5° C (Fig. 1), producing both subitaneous and resting eggs. On the other hand, Deevey (1960a) reports that 12° C is the lower limit of breeding in Delaware Bay. This difference could reflect acclimatization or genetic adaptation of individuals to the slightly differing thermal regimes of Vineyard Sound and Delaware Bay (see Bumpus, 1957). It has been shown for a wide variety of marine invertebrates that the tolerance limits for a number of biological functions differ between populations exposed to different environmental regimes (see Battaglia and Beardmore, 1978).

Whatever the factor(s) inducing the production of resting eggs by L. *aestica* from Vineyard Sound, it is shown that the response (*i.e.* initiation of dormancy) is first expressed in late August or early September. The intensity of the response increases as time progresses and the surface water temperatures decrease. This intensification is expressed as an increase in the percent production of resting

eggs and a decrease in subitaneous eggs. There is a strong correlation between temperature and the type of egg produced (Fig. 2b). However, temperature alone is not sufficient to account for the observed switch in egg production in early September (Fig. 1). The same temperatures (*i.e.* $18^{\circ}-20^{\circ}$ C) which are coincident with the production of resting eggs in September are observed in early summer when production is restricted to subitaneous eggs. Temperature may influence the type of egg produced, but it would appear that other factors are also involved.

The data show that subitaneous eggs are produced throughout the summer-fall period, and that resting eggs are produced only during the fall. There are clearly two egg types being produced in the fall. Subitaneous eggs produced at this time take longer to hatch (2–4 days) at 21° to 23° C than those produced in the summer (1–2 days).

The rate of embryonic development of copepod eggs is influenced by egg size, temperature, and environmental conditions experienced by the parents (McLaren, 1965; 1966; McLaren, Corkett, and Zillioux, 1969; Landry, 1975b; Hart and McLaren, 1978). For *L. aestiva* the observed difference in rate of development most probably reflects a maternal effect relating to the physiological condition of the female at the time of collection, as was shown for *Pseudocalanus* by Hart and McLaren (1978). No obvious size difference was observed between summer and fall subitaneous eggs of *L. aestiva* in the present study.

The fall subitaneous eggs of L. acstiva were detected by placing females collected at that time in an incubator at 19° C. If the subitaneous eggs produced by fall females were collected and held at temperatures equivalent to the ambient values in Vinevard Sound, their development would be retarded and they could be mistaken for resting eggs. On the other hand, fall eggs which do not hatch within 2 to 4 days at 21° to 23° C are to be regarded as resting eggs. As first demonstrated by Grice and Gibson (1975) resting eggs of L. aestiva remain viable for as long as 120 days at 5° C. It was suggested that a minimum chilling period of of 2 to 4 weeks at 5° C was required to break the dormant condition. The results of the present study show that if resting eggs are placed at 19° C without chilling, hatching will take place, but it takes longer and occurs sporadically. Chilling of resting eggs results in the reduction of time in actual diapause and synchronization of hatching. The diapause and hatching response of resting eggs of L. aestiva to different temperatures corresponds to several examples of insect and freshwater copepod diapause (Church and Salt, 1952; see Hutchinson, 1967; Stross, 1969b; Dean and Hartley, 1977a, b). The dormant state in these animals consists of a period of diapause followed by post-diapause development and then hatching (see Mansingh, 1971). During the diapause period, development is arrested and cannot resume even if conditions are favorable. Once the diapause is broken the individual is competent to resume development as soon as adverse conditions are terminated. For L. aestiva the actual breaking of diapause (reactivation) can take place at 19° C, but the process is faster at colder temperatures. Post-diapause development, however, is slower at reduced temperatures than at high temperatures. Therefore, individual chilled eggs terminate diapause at different times, and further development is retarded as long as the eggs remain chilled. The chilled eggs accumulate at a stage of readiness, and when exposed to higher temperatures are competent to proceed with development, resulting in synchronous hatching. On the other hand, individual resting eggs held continuously at 19° C terminate the diapause condition at different times (as the chilled eggs), but since the higher temperature is favorable for post-diapause development to proceed there is no accumulation of individuals at the boundary between diapause and post-diapause so that hatching is asynchronous.

Two attributes expressed by resting eggs are cold-hardiness and synchronous hatching. Resting eggs remain viable at 2° C for as long as 6 months (Grice and Gibson, 1975) whereas summer subitaneous eggs remain viable for no longer than 15 days (Grice, unpublished). The tolerance of fall subitaneous eggs has not been investigated. The cold resistance of resting eggs enables survival during the winter. Synchronization of development promotes the reproductive success of the first generation appearing in the early summer by ensuring that individuals will attain reproductive maturity at the same time. If hatching was sporadic, the number of mature individuals in the population at any one time might not attain a size sufficient for successful mating encounters to occur.

The effects of temperature on the maintenance and termination of resting eggs of L. aestica are similar to those observed for other plants and animals which overwinter. In the majority of cases for which diapause induction has also been investigated, photoperiod and temperature have been shown to be the two most important factors affecting the initiation of dormancy (Harvey, 1957; Stross and Hill, 1965; see Hutchinson, 1967; Stross, 1969a, b; Watson and Smallman, 1971; see Mansingh, 1971; de March, 1977; see Clutter, 1978). The pattern of egg production observed in this study would occur if a developmental stage perceived a cue which then triggered an irreversible sequence of events leading to one type of egg or another. The fact that resting egg production precedes the decline of surface water temperatures suggests that such a stimulus may exist. The successful species will evolve a dormancy response to a factor which closely parallels the stress (in this case temperature) but which itself is extremely stable (such as photoperiod). That two egg types are produced during the fall could be due to the extended survival of summer females which lay subitaneous eggs and the newly developed fall females programmed to produce resting eggs. Moreover, because no two individuals are alike, some females may respond to a weak stimulus, whereas others require a more intense exposure. In the spruce budworm (Harvey, 1957), diapause-free development occurs under a long day regime (i.e., greater than 15 hr of light) in a portion of the population. As day length is increased the number of non-diapausing insects increases, reaching 100% in continuous light. Universal diapause occurs when day length is less than 15 hr. Current progress in our laboratory indicates that short photoperiods (less than 12 hr light) are effective in inducing the production of resting eggs by laboratory-reared L. acstiva. This will be reported on at length in a subsequent paper.

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SUMMARY

The calanoid copepod, *Labidocera aestiva* was collected from Vineyard Sound, Massachusetts between July and December 1978. Adult size (cephalothorax and total body length) was inversely proportional to surface water temperature at the time of collection. The major switch from subitaneous to resting egg production occurred during late August to early September, but a small percent of subitaneous eggs continued to be produced throughout the fall. Resting eggs were cold-resistant and when chilled at 5° C hatched synchronously when warmed to 21° to 23° C. Individual resting eggs kept continuously at 19° C took longer to hatch and emergence was asynchronous. The resting eggs of *L. aestiva* appear to be in a state of diapause similar to many insects, and it is suggested that photoperiod is the primary cue inducing the production of resting eggs.

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